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Mol. Gen. Genet. (1992) 231 186-193 (Halter U. et al.)
Plant Cell (1990) 2 (5) 415-425 (Lee K. Y. et al.)

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(54) Abstract Title

In situ modification of plant genes

(57) A method of producing plants which exhibit an agronomically desirable trait comprises mutating or otherwise modifying in situ in a plant cell at least one gene and regenerating from a cell exhibiting the said trait fertile morphologically normal whole plants, and is characterised in that a polynucleotide is introduced into the plant cell, the said polynucleotide comprising at least one region which is substantially complementary to the gene, which gene when mutated or otherwise modified provides for the agronomically desirable trait, the region in the said polynucleotide containing at least one base mismatch in comparison with the like region in the said gene, so that the region in the said gene is altered by the DNA repair/replication system of the cell to include the said mismatch.

The agronomically desirable trait may be herbicide resistance and specific mutations to the EPSPS and PDS genes are disclosed. The polynucleotide may contain RNA and DNA sequences and may have areas of heteroduplex, homoduplex and unpaired bases.

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IMPROVEMENTS IN OR RELATING TO ORGANIC COMPOUNDS

The present invention relates to the production of plants which exhibit certain desirable agronomic traits and which are produced by a non-biological process not
5 obligatorily involving transformation or transgenesis (although these techniques can be used).

According to the present invention there is provided a method of producing plants which exhibit an agronomically desirable trait comprising mutating or otherwise modifying
10 *in situ* in a plant cell at least one gene which when modified is responsible for providing the said trait and regenerating from a cell exhibiting the said trait fertile morphologically normal whole plants, characterised in that a polynucleotide is introduced into the plant cell, the said polynucleotide comprising at least one region which is substantially complementary to at least one region in the gene, which gene region when mutated or otherwise modified provides for the agronomically desirable trait, the region in the said polynucleotide
15 containing at least one base mismatch in comparison with the like region in the said gene, so that the region in the said gene is altered by the DNA repair/replication system of the cell to include the said mismatch.

By "gene" is meant a polynucleotide comprising - contiguously - a sequence to which an RNA polymerase is capable of binding (promoter), an RNA encoding sequence and a
20 transcription termination sequence. At least one of the following regions of the gene may be mutated or otherwise modified: promoter, RNA encoding sequence or transcription terminator. In a preferred embodiment of the method a transcription enhancing region associated with the gene is mutated or otherwise modified *in situ*.

Whilst the said trait could be an improved resistance to insects and/or fungal or
25 bacterial infections, it is particularly preferred that the trait is herbicide resistance. The herbicides to which plants resulting from the method according to the invention are rendered resistant, or to which the said plants are tolerant or exhibit relatively improved resistance, are selected from the group consisting of paraquat; glyphosate; glufosinate; photosystem II inhibiting herbicides; dinitroanilines or other tubulin binding herbicides; herbicides which
30 inhibit imidazole glycerol phosphate dehydratase; herbicides which inhibit acetolactate synthase; herbicides which inhibit acetyl CoA carboxylase; herbicides which inhibit protoporphyrinogen oxidase; herbicides which inhibit phytoene desaturase; herbicides which

inhibit hydroxyphenylpyruvate dioxygenase and herbicides which inhibit the biosynthesis of cellulose.

Plants which are substantially "tolerant" to a herbicide when they are subjected to it provide a dose/response curve which is shifted to the right when compared with that provided by similarly subjected non tolerant like plants. Such dose/response curves have "dose" plotted on the x-axis and "percentage kill", "herbicidal effect" etc. plotted on the y-axis. Tolerant plants will require more herbicide than non tolerant like plants in order to produce a given herbicidal effect. Plants which are substantially "resistant" to the herbicide exhibit few, if any, necrotic, lytic, chlorotic or other lesions when subjected to the herbicide at concentrations and rates which are typically employed by the agrochemical community to kill weeds in the field. Plants which are resistant to a herbicide are also tolerant of the herbicide. The terms "resistant" and "tolerant" are to be construed as "tolerant and/or resistant" within the context of the present application.

The skilled man will appreciate that the plant material in which the *in situ* modification is performed may have been prior transformed with a gene providing for resistance to insects, fungi, and/or herbicides, or with a gene capable of providing plants regenerated from such material with, for example, an increased capacity to withstand adverse environmental conditions (improved drought and/or salt tolerance, for example) in comparison with plants regenerated from non-transformed like material.

At least one region of the polynucleotide may consist of RNA. The polynucleotide other than that comprised by the said at least one region may consist of DNA. The polynucleotide may consist of between about 30 and 250 nucleotides. In a more preferred embodiment of the polynucleotide it consists of between 50 and 200 nucleotides.

The protein encoding region of the gene may encode an enzyme selected from the group consisting of EPSPS, GOX, PAT, HPPD, ACC, ALS, BNX and protox and known mutated or variant forms thereof. In particular, the said gene may encode an EPSPS enzyme as depicted, for example, in SEQ ID Nos. 1 or 10. It is preferred that the EPSPS enzyme has least the residues Thr, Pro, Gly and Ala at positions corresponding to 174, 178, 173 and 264 with respect to the EPSPS depicted in SEQ ID No. 2, and that the said mismatch results in at least one of the following modifications in the EPSPS enzyme in comparison with the native sequence:

- (i) Thr 174 - Ile
- (ii) Pro 178 - Ser
- (iii) Gly 173 - Ala
- (iv) Ala 264 - Thr

5 wherein (i) Thr 174 occurs within a sequence comprising contiguously Ala -Gly-Thr-Ala-Met; (ii) Pro 178 occurs within a sequence comprising contiguously Met-Arg-Pro-Leu-Thr; (iii) Gly 173 occurs within a sequence comprising contiguously Asn-Ala-Gly-Thr-Ala; and (iv) Ala 264 occurs within a sequence comprising contiguously Pro-Leu-Ala-Leu-Gly.

Alternatively, and/or additionally, the mismatch may result in replacement of the
10 terminal Gly residue within the sequence motif Glu-Arg-Pro-AA1-AA2-AA3-Leu-Val-AA4-AA5-Leu-AA6-AA7-AA8-Gly- in a region of the EPSPS enzyme corresponding to that spanning positions 202 to 216 in SEQ ID No. 2 by either an Asp or Asn residue.

The plant cell to which the method of the invention is applied may be a cell of a plant selected from the group consisting of canola, sunflower, tobacco, sugar beet, cotton, maize,
15 wheat, barley, rice, sorghum, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, cabbage, onion, soya spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and forage grasses, flax and oilseed rape, and nut producing plants insofar as they are not already specifically mentioned

The plant cell may be converted into a protoplast prior to the *in situ* mutation or
20 modification of the gene - or transcriptional enhancing regions associated therewith - which when modified provides for the agronomically desirable trait.

The invention further includes plants which result from the method disclosed herein, as well as the progeny and seeds of such plants, and plant material derived from such plants, progeny and seeds.

25 The invention still further includes a method of selectively controlling weeds in a field, the field comprising plants as disclosed in the preceding paragraph and weeds, the method comprising application to the field of a herbicide to which the said plants have been rendered resistant. Insecticidally effective amounts of insecticides and/or fungicidally effective amounts of fungicides may optionally be applied to the said plants, preferably after
30 the herbicide has been applied to the field.

The invention will be further apparent from the following description taken in conjunction with the associated sequence listing.

SEQ ID No. 1 shows the cDNA from *Petunia* encoding an EPSPS enzyme. Nucleotides 28 to 243 encode the transit peptide responsible for targeting the EPSPS enzyme encoded by nucleotides 244 to 1578 to the chloroplast. SEQ ID No. 2 shows the translational product of the sequence depicted in SEQ ID No. 1. Protein having the sequence of amino acid residues 1 to 72 constitutes the chloroplast transit peptide; protein having the sequence of amino acids 73 to 516 constitutes the EPSPS enzyme. SEQ ID Nos 3 and 4 depict peptides encoded by sequences (SEQ ID Nos 5 and 7) within exons 2 and 4 respectively of the *Brassica napus* EPSPS gene. Sequence ID Nos. 6 and 8 are mixed ribo-deoxyribonucleic acid sequences which are capable of forming duplexes with the sequences depicted in SEQ ID Nos. 5 and 7 respectively. SEQ ID Nos. 28 and 29 are sequences which are comprised by the sequences depicted in SEQ ID Nos. 5 and 7 respectively. SEQ ID Nos. 9 and 10 depict respectively (i) the genomic DNA from *Brassica napus* which encodes a spliced RNA encoding an EPSPS enzyme, and (ii) the amino acid sequence of the said *Brassica* EPSPS enzyme. SEQ ID Nos 11 - 27 depict mixed oligonucleotides (ie containing both ribo and deoxyribonucleotides) comprising sequences (marked with asterixes in the reiteration of the sequences in the corresponding Examples) capable of causing mutations in the gene to which the oligonucleotide is targeted. The oligonucleotides depicted in SEQ ID Nos 11 to 27 are all designed to cause plant material into which they are incorporated to become resistant to herbicides, such as glyphosate and chlorsulfuron, by causing the gene encoding the proteinaceous target for the herbicide to become mutated so that the target is no longer sensitive to the herbicide. Should there be a discrepancy between the sequences depicted in the sequence listings and those corresponding sequences depicted in the Examples, the Example sequences are definitive. In the Examples sequences depicted in lower case are RNA and those in upper case are DNA.

Methods

Polynucleotides Mixed ribo-deoxyribonucleic acids are synthesised by synthetic and semisynthetic methods known to those skilled in the art (for example Scaringe, S.A. et al (1990), Nucleic Acids Research 18:5433-5441; Usman, N. et al (1992) Nucleic Acids Research 20:665-6699 and Swiderski, P.M. et al (1994) Anal. Biochem. 216:83-88. Eric B. Kmiec (1996) United States Patent 5,565,350). Mixed ribo-deoxyribonucleic acids are synthesised using natural nucleotides, or, in some cases, preferably with 2'-O methylated ribonucleotides. Additionally or alternatively the phosphodiester bonds of the nucleic acid

are replaced by phosphorothiodiesters or methylphosphonodiesters. Additionally or alternatively arabinose-containing nucleotides are also used.

A duplex nucleic acid in which deoxyribonucleotides and ribonucleotides correspond with each other is termed a hybrid-duplex. When two strands form a region of duplex
5 nucleic acid for less than all of their bases the resultant molecule is termed a heteroduplex. Two strands of a duplex can be linked by an oligonucleotide linker region to form a single polymer. The bases in the linker region are not Watson-Crick paired. A heteroduplex in which the first and second strands are portions of a single polymer is termed a hairpin duplex.

10 The mixed ribo-deoxyribonucleic acid useful in the present invention has at most one 3' end and one 5' end. It is constructed to contain at least one region of at least one or more - usually three to four - bases that are not Watson-Crick paired. These unpaired regions form linker regions between two strands of Watson-Crick paired bases. It is preferred that the bases of the linker regions are deoxyribonucleotides.

15 In a preferred embodiment, the mixed ribo-deoxyribonucleic acid is constructed having two linkers arranged a) such that substantially all of the remaining bases are Watson-Crick paired and b) such that the 3' and 5' ends of the polymer are Watson-Crick paired to adjacent nucleotides of the complementary strand. These can be ligated to form a single continuous circular mixed ribo-deoxyribonucleic acid polymer.

20 In the present invention, the mixed ribo-deoxyribonucleic acid is used for the purpose of specifically introducing alterations (a mutation) into a target gene. The genetic site of alteration is determined by selecting a portion of the mixed ribo-deoxyribonucleic acid to have the same sequence as (to be homologous with) the sequence of the target site, hereinafter termed a homologous region. The area of differences between the sequence of
25 the mixed ribo-deoxyribonucleic acid and the target gene is termed the heterologous region. Preferably there are two homologous regions in each mixed ribo-deoxyribonucleic acid flanking an interposed heterologous region, all three regions being present in a single continuous duplex nucleic acid. Furthermore each homologous region contains a portion of hybrid duplex nucleic acid. The portion of each hybrid-duplex is at least 4 base pairs,
30 preferably 8 base pairs and more preferably about 20 to 30 base pairs. A dinucleotide base pair of homo-duplex may be placed within a region of hybrid duplex to allow ligation of the

3' and 5' ends to each other. The total length of the two homologous regions is at least 20 base pairs and preferably is between 40 and 60 base pairs.

A region of homo-duplex can be disposed between the hybrid-duplex/ homologous regions of the vector. The interposed homo-duplex can contain the heterologous region.

5 When the heterologous region is less than about 50 base pairs and preferably less than about 20 base pairs, the presence of an interposed homo-duplex is optional. When the heterologous region exceeds about 20 base pairs, an interposed homo-duplex is preferred.

The change to be introduced into the target gene is encoded by the heterologous region. The change to be introduced may be a change in one or more bases of the target gene
10 sequence or the addition of one or more bases.

Design of polynucleotides to achieve in situ mutagenesis of EPSP synthase in

Brassica napus variety Westar.

It is known that the combination of mutations G101A and A192T in a Petunia EPSPS can provide for resistance to glyphosate, whilst maintaining a low Km for PEP. The equivalent residues in the sequence of the *B. napus* enzyme are (1) the
15 second glycine occurring within the sequence LGNAGTAMRPLT (SEQ ID No. 3) where this G is amino acid 173 wherein amino acid 1 is the starting methionine of the transit peptide and (2) the third alanine occurring within the sequence MAAPLALGDVEI (SEQ ID No. 4) and consequential having the residue number 264.

The glycine residue occurs within exon 2 (part of which is shown below and is
20 depicted as SEQ ID No. 5), the DNA coding sequence in the region being:

L G N A G T A M R P L T

ATTGAGTTGTACCTTGGGAATGCAGGAACAGCCATGCGTCCACTCACCGCTGCA

An example of the desired mutation is GGA ---> GCA

The mixed ribo-deoxyribonucleic acid designed to elicit this change includes, for
25 example, on one of its strands, a sequence comprising mainly of RNA which is complementary to all or part of the above DNA sequence. This RNA is interposed by a short region of DNA also complementary with the corresponding region of the above DNA sequence except for the inclusion of the specific mismatch of having a guanosine base opposite the guanosine base within the target GGA codon. A suitable mixed ribo-
30 deoxyribonucleic acid could thus include all or part of the following sequence (depicted as SEQ ID No. 6 in the sequence listing). Note that RNA sequence is marked in bold.

TTGTACCTTGGGAATGCAGGAACAGCCATGCGTCCACTC
AACAUGGAACCCUACGTCGTTGUCGGUACGCAGGUGAG

The corresponding alanine residue occurs within exon 4 (part of which is shown below and is depicted as SEQ ID No. 7).

5 M A A P L A L G D V E I
ACTGCCCTCCTCATGGCAGCTCCTTTAGCTCTTGGAGACGTGGAGATTGAGATCATT

An example of the desired mutation is GCT ---> ACT. The mixed ribo-
deoxyribonucleic acid designed to elicit this change includes, for example, on one of its
strands, a sequence comprising mainly of RNA which is complementary to all or part of the
10 above DNA sequence. This RNA is interposed by a short region of DNA also
complementary with the corresponding region of the above DNA sequence except for the
inclusion of the specific mismatch of having a thymine base opposite the guanosine base
within the target GCT codon. The desired polynucleotide thus includes all or part of the
RNA sequence depicted below and in SEQ ID No. 8. Note that RNA sequence is marked in
15 bold.

TCCTCATGGCAGCTCCTTTAGCTCTTGGAGACGTGGAGATT
AGGAGUACCGUCGAGGAAATTGAGAACCUCUGCACCUCUAA

Besides the examples detailed above there will of course be many other specific
changes which could be introduced into those sequences which regulate gene expression and
20 for which polynucleotides can easily be designed by methods directly analogous to that
described above and which, for example, could be useful to achieve increased expression of
EPSPS. The skilled man will appreciate that many methods could be used to specify those
changes potentially useful for increasing the expression of EPSPS. For example:

(1) The skilled man will be aware of instances of resistance to glyphosate having
25 occurred in both field populations of weeds (e.g Australian lolium) and upon continuous
selection of cultured plant cells (e.g. Hollander-Czytko et al (1988) in Plant Mol. Biol, 11,
215-220; Hollander-Czytko et al (1992) Plant. Mol. Biol. 20, 1029-1036) or, for example,
cultivars of birdsfoot trefoil (Boerboom et al (1990) Weed. Sci., 38, 463-467) upon
glyphosate. In the latter two cases selection was shown to have resulted in a significant
30 increase in expression of EPSP synthase. In the example of the work on cell cultures of
Corydalis sempervirens (Hollander-Czytko et al (1988) in Plant Mol. Biol, 11, 215-220) a
30-40 fold increase in the cellular content of EPSP synthase and an 8-12 fold increase in
transcript levels was observed. There was no amplification of the EPSP synthase gene.

It is a routine matter in all of the above examples using methods known to the skilled man to isolate cDNA encoding the EPSP synthases, to use these cDNA's as probes to identify clones from genomic libraries and to sequence the corresponding EPSP synthase genes and their 5' upstream and 3' downstream regions. Alternatively, genomic sequences may be isolated directly using heterologous probes and/or combinations of degenerate and inverse PCR. By comparing the sequences so obtained from 'high EPSP synthase expression' lines of plants, cultivars or plant cells with the appropriate unselected controls the specific mutation(s) responsible for conferring high expression of EPSP synthase will be identified.

(2) Another example of a suitable method for identifying mutations potentially useful for increasing the expression of EPSP synthase is to directly select various lines of cultured plant cells or protoplasts from plant species of interest (e.g. *Brassica napus*) on increasing concentrations of glyphosate. This can be done with or without the addition of a suitable chemical mutagen. Glyphosate-tolerant lines so obtained are analysed for expression of EPSP synthase, for the level of translatable EPSP synthase gene transcript (e.g. by Northern analysis) and for possible amplification of the EPSPS gene (e.g. by Southern and dot blot analysis). Cell lines of particular interest would be those where EPSP synthase was overexpressed and where this increase could not be accounted for through gene amplification. Identification of the specific mutation(s) responsible for conferring high expression of EPSP synthase are then identified as described in (1) above.

(3) A further example of a method useful to specify mutations causing high expression of EPSPS comprises (a) subcloning the plant EPSP synthase promoter, 5' upstream sequence region, translational start region and sequence encoding the N-terminus region of EPSP synthase into a translational fusion construct directing the synthesis of a suitable and easily measurable reporter gene such as (Beta glucuronidase) (b) further cloning this into a shuttle vector containing an origin for replication in *E.coli* and also designed for site specific integration into the yeast genome (YIP), or the genome of any other suitable test cell, such that integration into a specific location can be positively selected, by for example, complementation of an auxotrophic mutation. A library of many variants specifically within the promoter and 5' upstream region of the so-designed shuttle vector is then created by mutagenesis through, for example, Mn²⁺-poisoned PCR of the region and maintained in *E.coli*. Members of the library are then tested by transformation into yeast. The best

expressers in yeast are identified by increased expression of the reporter gene. The integrated DNA from these high expresser lines is then extracted, sequenced and compared with the original sequence in order to identify those specific mutation(s) which conferred increased expression. Such mutations may affect conserved domains within the promoter
5 which bind the transcriptional activators required for gene expression. Studies of this sort may teach those skilled in the art to modify the equivalent conserved regions in other crop plant species, thus enabling the technology to be applied broadly.

The polynucleotides comprising the RNA sequences disclosed above are transfected into protoplasts of *Brassica napus* which are then cultured and subjected to the herbicide
10 glyphosate at concentrations which are sufficient to kill like protoplasts which have not been transfected and like protoplasts which have been transfected but with a polynucleotide not comprising regions designed to elicit a mutation in the *Brassica* genome. Those transfected protoplasts which survive the herbicide at concentrations which kill the control protoplasts are regenerated into plants using known means. The increased resistance to the herbicide of
15 the thus regenerated plants is inherited in a Mendelian manner amongst the progeny of these plants.

The skilled man will appreciate that the invention is not limited to that specifically described above in respect of the production of glyphosate resistant *Brassica napus*. For plant species for which the EPSP synthase gene sequence(s) are already available on public
20 databases the RNA and DNA elements of the polynucleotides can easily be designed by a method directly analogous to that described for *B. napus*. Polynucleotides comprising these RNA and DNA elements can then be introduced into regeneratable plant material from other species. Moreover, the skilled man is capable of designing:

(i) polynucleotides for the *in situ* mutagenesis of the DNA bases flanking the
25 translational start site to improve post transcriptional efficiency of expression of EPSP synthase in plants, for example *Brassica napus* variety *Westar*. The consensus sequences for the regions immediately surrounding the translational start sites in animals (M Kozak, 1986, Cell, 44, 283-292) and plants (G Heidecker and J Messing, 1986, Ann. Rev. Plant Physiol., 37, 439-466; V Pautot et al., 1989, Gene, 77, 133-140) have been described. It is therefore
30 possible that improved levels of expression of the native *B. napus* EPSP synthase gene may be improved *in situ* by designing mixed ribo-deoxyribonucleic oligonucleotides to make the

desired mutational changes, at positions -3 and + 6 as shown below. Note that conserved consensus sequences are underlined.

	-4	-3	-2	-1	+1	+2	+3	+4	+5	+6
B. napus	<u>A</u>	T	C	A	<u>A</u>	<u>T</u>	<u>G</u>	<u>G</u>	<u>C</u>	G
Consensus	<u>A</u>	<u>A</u>	C	A	<u>A</u>	<u>T</u>	<u>G</u>	<u>G</u>	<u>C</u>	<u>T</u>

It will be obvious to those skilled in the art that this approach need not be confined to the EPSP synthase gene from B. napus, but may be applied to any plant species in which an

5 increase in expression of the target gene is sought.

ii) polynucleotides for the *in situ* mutagenesis of the DNA bases to achieve an increase in transcriptional efficiency of expression of EPSP synthase. An approach similar to that described above may be adopted to achieve an enhancement in the rate of transcription of EPSP synthase genes by mutating bases at the "TATA" box region upstream from the transcription start point, and at the transcription start point itself. Identification of the transcription start point is identified using techniques, such as primer extension analysis, known to those skilled in the art. The "TATA" box is generally found 16-54 bases upstream of the transcriptional start. Consensus sequences have been published for plant transcription start point (V Pautot et al., 1989, Gene, 77, 133-140)

15 Plant Consensus CTCATCA

and "TATA" box regions (V Pautot et al., 1989, Gene, 77, 133-140)

Plant Consensus TCACTATATATAG

In both cases highly conserved bases are underlined. Comparisons between the consensus and native sequences of target EPSP synthase genes will enable bases suitable for mutational change to be identified.

20 (iii) polynucleotides for *in situ* mutagenesis to alter expression of EPSP synthase in plants, for example *Brassica napus* variety *Westar*.

Such designed polynucleotides can be introduced into totipotent plant material by known means which is then regenerated into plants which are subjected to a selection procedure to isolate those that exhibit the desired trait.

25 The skilled man will appreciate that directly analogous methods to those described above for EPSP synthase and glyphosate could be applied to other combinations of selecting herbicide and target gene where the aim is to specify mutations conferring over-expression.

The invention will be further apparent from the following Examples. Throughout the Examples the expression "selecting concentrations" of herbicide is present. By this is meant a concentration of herbicide which is sufficient to kill non-transformed material, or material which otherwise does not contain the oligonucleotides which are contained within like experimental material. The skilled man will know what those concentrations are having regard to the specific circumstances relating to his particular germplasm, transformation protocols and the expected variation between replicate procedures. The oligonucleotides shown below (SEQ ID Nos 11 to 27) are all synthesised according to Yoon *et al.* (1996). In each of the Examples where the constructs contain bases depicted in lower case, the sequence comprising such bases is to be understood as being RNA, and sequences comprising bases depicted in upper case as being DNA.

Example 1 This Example demonstrates the production of corn (maize) which is resistant to the herbicide chlorsulfuron.

```

15                                     *
      TGC GCG gauacuagggATTACcacc+ccgaaT
      T                                     T
      T                                     T
20      TCGCGC CTATGATCCCTAATGGTGGGGCTTT
           3'5'

```

The above oligonucleotide (SEQ ID No. 11) conveniently may be introduced into corn using silicon carbide whiskers, pollen harbouring the oligonucleotide or *via* pollen tubes.

Whiskers The so called whiskers technique is performed essentially as described by Frame *et al.*, (1994 Plant J. 6 941 -948). The oligonucleotide (1-100 µg) depicted in SEQ ID No.11 is added to the whiskers and used to transform A188 x b73 cell suspensions. The oligonucleotide(s) may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. Plant regeneration is performed using selective concentrations of chlorsulfuron in place of bialophos. Plants are transferred to pots and matured in the green house. Kernal from these plants are germinated in soil and sprayed with a selecting concentration of chlorsulfuron 9 to 14 days post emergence.

Pollen transformation Maize pollen is bombarded with gold particles by techniques known to the skilled man. Gold particles are coated with the oligonucleotide depicted in SEQ ID No. 11. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence.

Suitable bombardment methods vary in precise detail but the basic procedure is well known to the skilled man and it is thus not necessary to describe it here. Bombarded pollen is applied to receptive silks of detassled plants. Sufficient replicas are performed to pollinate a large number of plants (typically up to 500). Progeny of the plants are screened for chlorsulfuron resistant members of the population by spraying with selecting concentrations of chlorsulfuron.

Pollen tube mediated transformation Emasculated corn plants are used. Wild type pollen is applied to pollination receptive silks. After between 30 min to 6 hours the silks are cut to within one cm of the base. The above SEQ ID No. 11 oligonucleotide (1-100 µg/ 10 µl in TE) is applied to the cut surface using a 1 ml syringe and needle such that the surface is completely covered. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. The plants are then grown in a green house with an initial humidity of about 75 %. Progeny of the plants are screened for chlorsulfuron resistant members of the population by spraying with selecting concentrations of the herbicide.

Plants derived from material into which the oligonucleotides have been incorporated are resistant, more resistant or tolerant to the herbicide, when compared to plants derived from material not containing the said oligonucleotide.

Example 2 This Example demonstrates the production of *Arabidopsis thaliana* which is resistant to the herbicide glyphosate (and suitable salts thereof). The following oligonucleotides (depicted as SEQ ID Nos 12 to 16 in the sequence listing) are prepared using standard technology.

T to I

*

```

      T GCGCG cauuacguccTTATCguuacgcagg T
5      T                                     T
      T                                     T
      T CGCGC GTAATGCAGGAATAGCAATGCGTCC T
          3'5'      (SEQ ID No. 12)
```

10 T to I2

*

```

      T GCGCG cauuacgtccTTATCguuacgcaag T
      T                                     T
      T                                     T
15     T CGCGC GTAATGCAGGAATAGCAATGCGTTC T
          3'5'      (SEQ ID No. 13)
```

P to S

*

```

20     T GCGCG ugucguuacgCAAGTgaauggcgac T
      T                                     T
      T                                     T
      T CGCGC ACAGCAATGCGTTCACCTACCGCTG T
25     3'5'      (SEQ ID No. 14)
```

P to S 2

*

```

30     T GCGCG uaucguuacgCAAGTgaauggcgac T
      T                                     T
      T                                     T
      T CGCGC ATAGCAATGCGTTCACCTACCGCTG T
35     3'5'      (SEQ ID No. 15)
```

* *

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40     T GCGCG cauuacguccTTATCguuacgCAAGTgaguggcgac T
      T                                     T
      T                                     T
      T CGCGC GTAATGCAGGAATAGCAATGCGTTCACCTACCGCTG T
          3'5'      (SEQ ID No. 16)
```

45 These oligonucleotides are introduced into *Arabidopsis* by microprojectile bombardment or protoplast uptake.

Bombardments *Arabidopsis* is transformed essentially using a modified procedure as described by Seki *et al.* ((1991) Appl. Microbiol. Biotechnol. 36 228-230). *Arabidopsis thaliana* genotype C24 seeds are surface sterilised and sown on B-5 medium

(Gamborg *et al.* , 1968) solidified with 0.6 % agarose. The plants are grown aseptically for 4 - 6 weeks under 16 h light 8 h dark at 26 °C. Roots are harvested and cut into sections that are 0.5 - 1.0 cm long and placed onto a filter paper on medium containing B5 salts and vitamins, 3 % sucrose, 0.5 mg/ml 2,4-dichlorophenoxyacetic acid, 0.05 mg/l kinetin and 0.8 % agarose (0.5 - 0.05 medium). After two to five days the roots are ready for bombardment. Gold particles (10 mg; Hereus, 0.4-1.2 µm diameter) are coated with 1 - 100 µg of oligonucleotide as follows. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. The particles are suspended in 1 ml of absolute ethanol and incubated for three hours at room temperature then stored at -20°C. Twenty to thirty-five µl of sterile resuspended particles are collected by centrifugation in a microcentrifuge. The particles are washed with one ml of sterile distilled water and re-collected by centrifugation. Microprojectiles are then resuspended in 30 µl oligonucleotide solution (1 -100 µg), 25 µl of 1M CaCl₂ is added followed by 10 µl of 0.1 M spermidine (free base). The mixture is incubated on ice for 10 minutes. 1 -10 µl of this solution is used per bombardment. A suitable mixture or combination of oligonucleotides is introduced into plant material either simultaneously or sequentially. If the oligonucleotides are introduced sequentially, they must be introduced in such a way that the mutation governed by the first oligonucleotide is not negated by the mutation governed by a subsequently introduced oligonucleotide. For example, if the oligonucleotide depicted by SEQ ID No. 12 is introduced first, the oligonucleotide depicted by SEQ ID No. 15 should be used subsequently. Alternatively, a single oligonucleotide comprising regions providing for multiple mutations (such as that depicted in SEQ ID No. 16) may be used.

The roots are bombarded with oligonucleotide-coated particles by a helium-driven biolistics PDS 1000 system (BioRad) with a 300 mm Hg vacuum. The levels between the rupture disk and the macrocarrier and the macro-carrier and sample are varied for maximal transformation efficiency. Rupture disks of between 1000 and 2000 psi are used. Two suitable oligonucleotides are introduced into *Arabidopsis* plant material either simultaneously or sequentially. For simultaneous transformation the oligonucleotides are used in equal molar concentrations and may be introduced into the material by multiple firings into the same tissue. For sequential transformation the roots receive at least one

bombardment with each oligonucleotide but multiple firings of each oligonucleotide are used if necessary to optimise transformation efficiencies.

After the bombardments the plant material is transferred to 0.5 - 0.05 medium and incubated at 26°C for one to 5 days. Regeneration of transformed material into *Arabidopsis* plants is performed as Seki *et al* 1991 with the exception that kanamycin or gentamycin are not included in any of the media. Instead the transformed material is selected by its resistance or tolerance to glyphosate, present in the selection medium at a concentration sufficient to kill control material which has been subjected to a like transformation procedure with the *proviso* that it does not contain the oligonucleotides specified above.

DNA uptake by protoplasts incubated in PEG The protocol of Dam *et al.* (1989 Mol Gen. Genet 217 6-12) is followed. Instead of using linearised plasmid DNA in the transformation an equal molar ratio mix of the two oligonucleotides (SEQ ID Nos 12 and 15) are used (1- 100 µg) with 50 -100 µg calf thymus carrier DNA. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. Glyphosate selection instead of hygromycin selection is applied at the same stage during callus formation. The concentration of glyphosate used is varied to give optimum selection of transformed *Arabidopsis* plants, but is determined by reference to suitable control experiments.

Plants derived from material into which the oligonucleotides have been incorporated are resistant, more resistant or tolerant to the herbicide, when compared to plants derived from material not containing the said oligonucleotide.

Example 3 This Example demonstrates the provision of glyphosate resistant *Brassica napus*

T to I

*

```

30  T GCGCG ccuacguccTTATCgcuacgcagg T
    T
    T
    T CGCGC GGAATGCAGGAATAGCCATGCGTCC T
      3'5' (SEQ ID No. 17)
  
```

T to I 2

```

      *
T GCGCG ccuuacgtccTTATCgcuacgcaag T
T                                     T
5  T                                     T
T CGCGC GGAATGCAGGAATAGCCATGCGTTC T
      3'5'      (SEQ ID No. 18)

```

10 P to S

```

      *
T GCGCG ugucgguacgCAAGTgaguggcgac T
T                                     T
T                                     T
15 T CGCGC ACAGCCATGCGTTCCTCACCCTG T
      3'5'      (SEQ ID No. 19)

```

P to S 2

```

      *
T GCGCG uaucgguacgCAAGTgaguggcgac T
T                                     T
T                                     T
25 T CGCGC ATAGCCATGCGTTCCTCACCCTG T
      3'5'      (SEQ ID No. 20)

```

```

      *      *
30 T GCGCG ccuuacguccTTATCgcuacgCAAGTgaguggcgac T
T                                     T
T                                     T
T CGCGC GGAATGCAGGAATAGCCATGCGTTCCTCACCCTG T
      3'5'      (SEQ ID No. 21)

```

35 These oligonucleotides are designed to target the *Brassica napus* EPSPS gene. The oligonucleotides provide for two changes in the sequence of the protein encoded by the gene, viz. at T102 and P106 of the Brassica mature enzyme such that the mutant gene (via an altered protein product) confers resistance to glyphosate.

40 The oligonucleotides are introduced into *Brassica napus* using known methods which includes microprojectile bombardment or uptake of DNA by protoplasts.

Bombardments Seeds of *B.napus cv Westar* are surface sterilised in 1% sodium hypochlorite for 20 minutes. The seeds are then washed in sterile water three times and planted at a density of about 10 seeds per plate on Murashige Skoog (MS) minimal organics medium (GibcoBrl) with 3% sucrose and 0.7% phytagar (Gibco) pH 5.8. Seeds are
45 germinated at 24 °C in 16 h light/8h dark. After five days the cotyledons are excised in such a

way that they include approximately 2 mm of petiole at the base. Care is taken to exclude the apical meristem. The excised cotyledons are placed on MS medium, 3 % sucrose and 0.7 % phytagar enriched with 20 μ M bezyladenine with the petioles imbedded to a depth of 2 mm in the medium at a density of about ten cotyledons per plate.

- 5 Gold particles (10 mg; Hereus, 0.4-1.2 μ m diameter) are coated with 1 - 100 μ g of oligonucleotide (SEQ ID No. 22 for example, or SEQ ID Nos. 18 and 20) in plant cells. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. The particles are suspended
10 in 1 ml of absolute ethanol and incubated for three hours at room temperature then stored at -20oc. Twenty to thirty five μ l of sterile resuspended particles are collected by centrifugation in a microcentrifuge. The particles are washed with one ml of sterile distilled water and re-collected by centrifugation. Microprojectiles are then resuspended in 30 μ l solution (containing oligonucleotides depicted in SEQ ID Nos. 18 and 20, for example in an amount
15 of about 1 -100 ug). 25 μ l of 1M CaCl₂ is added followed by 10 μ l of 0.1 M spermidine (free base). The mixture is incubated on ice for 10 minutes. 1 -10 μ l of this solution is used per bombardment.

The cotyledons are bombarded with oligonucleotide-coated particles by a helium-driven biolistics PDS 1000 system (BioRad) with a 300 mm Hg vacuum. The levels between
20 the rupture disk and the macrocarrier and the macro-carrier and sample are varied for maximal transformation efficiency. Rupture disks of between 1000 and 2000 psi are used. The two oligonucleotides are introduced into the *Brassica* plant material either simultaneously or sequentially. For simultaneous transformation the oligonucleotides are used in equal molar concentrations and may be introduced into the explant by multiple
25 firings into the same tissue. For sequential transformation the explants receive at least one bombardment with each oligonucleotide but multiple firings of each oligonucleotide are used as necessary to optimise transformation efficiencies.

- After bombardment the explants are placed onto regeneration medium comprising MS medium supplemented with 20 μ M benzyladenine, 3% sucrose 0.7% phytagar pH 5.8.
30 After 2 - 5 days the cotyledons are transferred to plates containing the same media but including selective concentrations of glyphosate. The petioles remain embedded in the media. The explants are left for 2 - 6 weeks and then transferred onto MS medium

supplemented with 3 % sucrose, 0.7% phytagar pH 5.8 and selecting concentrations of glyphosate. One to three weeks later surviving shoots are transferred to rooting media which comprises MS medium, 3% sucrose, 2 mg/ml indole butyric acid, 0.7% phytagar with no glyphosate. Once roots are visible the plants are transferred to pots and propagated in the greenhouse.

Protoplast uptake The method of Golz *et al.* ((1990) Plant Mol Biol 15 475 - 483) is followed. *Brassica napus* genotype H1 is used. Instead of using plasmid DNA in the transformation an equal molar ratio mix of the two oligonucleotides (SEQ ID Nos 18 and 20) are used (1- 100 µg) and 20 -100 µg calf thymus carrier DNA. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. Glyphosate selection instead of hygromycin selection is applied at the same stage during callus formation. The concentration of glyphosate used is varied to give optimum selection of transformed *Brassica* plants.

Plants derived from material into which the oligonucleotides have been incorporated are resistant, more resistant or tolerant to the herbicide, when compared to plants derived from material not containing the said oligonucleotide.

Example 4 This Example demonstrates the provision of corn resistant to the herbicide glyphosate (and salts thereof).

T to I

**

```

  T GCGCG ccuacgaccTTAGCGuuacgccgqua T
25 T
  T CGCGC GGAATGCTGGAATCGCAATGCGGCCAT T
    3' 5'      (SEQ ID No. 22)
```

**

```

  T GCGCG ccuacgaccTTAGCGuuacgccagua T
30 T
  T CGCGC GGAATGCTGGAATCGCAATGCGGTCAT T
35 3' 5'      (SEQ ID No. 23)
```

P to S

```

      *
      T GCGCG gacguuacgCCAGTaacugucgucg T
      T                                     T
5      T                                     T
      T CGCGC CTGCAATGCGGTCATTGACAGCAGC T
          3'5'      (SEQ ID No. 24)
  
```

P to S 2

```

      *
      T GCGCG agcguuacgCCAGTaacugtcgucg T
      T                                     T
      T                                     T
      T CGCGC TCGCAATGCGGTCATTGACAGCAGC T
15          3'5'      (SEQ ID No. 25)
  
```

```

      **      *
      T GCGCG ccuuacgaccTTAGCGuuacgCCAGTaacugucgucg T
20      T                                     T
      T                                     T
      T CGCGC GGAATGCTGGAATCGCAATGCGGTCATTGACAGCAGC T
          3'5'      (SEQ ID No. 26)
  
```

25 These oligonucleotides which are designated as SEQ ID Nos 22-26 in the sequence listing and which are produced by means known to the skilled man, may be introduced into corn using silicon carbide whiskers, pollen harbouring oligonucleotides or via pollen tubes.

... **Silicon carbide whiskers** This transformation is performed essentially as described by Frame *et al.* (1994 Plant J. 6 941-948). The oligonucleotide depicted as SEQ ID No 26 (1-
30 100 µg) is added to the whiskers and used to transform A188 x B73 cell suspensions. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. Plant regeneration is performed using selective concentrations of glyphosate in place of bialophos. Plants are
35 transferred to pots and are then matured in the green house. Caryopsis from these plants are germinated in soil and sprayed with a selecting concentration of glyphosate 9 to 14 days post emergence.

Pollen transformation. Maize pollen is bombarded with gold particles (essentially as described in the above Examples) coated with a mixture of the above oligonucleotides (SEQ
40 ID Nos 23 and 25). The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such

that recombination is catalysed between the oligonucleotide and the target sequence.

Bombarded pollen is applied to receptive silks of detassled plants. Sufficient replicas are performed to pollinate a large number (typically up to 300) of plants. Progeny of the plants are screened for glyphosate resistant members of the population by spraying with selecting concentrations of glyphosate.

Pollen tube mediated transformation Emasculated corn plants are used. Wild type pollen is applied to pollination receptive silks. After between 30 min to 6 hours the silks are cut to within one cm of the base. Suitable mixtures of the above oligonucleotides (1-100µg/10 µl in TE) are applied to the cut surface using a 1 ml syringe and needle such that surface is completely covered. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. The plants are then grown in a green house with an initial humidity of about 75 %. Progeny of the plants are screened for glyphosate resistant members of the population by spraying with selecting concentrations of glyphosate.

Plants derived from material into which the oligonucleotides have been incorporated are resistant, more resistant or tolerant to the herbicide, when compared to plants derived from material not containing the said oligonucleotide.

Example 5 This Example demonstrates the provision of tomato plants resistant to a bleaching herbicide designated as R390244.

*

```

T GCGCC agcguaacuuGTCGAaagaagucca T
T                                     T
T                                     T
25 T CGCGC TCGCATTGAACAGCTTTCTTCAGGT T
    3' 5'      (SEQ ID No. 27)
```

This oligonucleotide (SEQ ID No. 27) is designed to target the codon for arginine 307 of the tomato phytoene desaturase (PDS) gene and introduce a mutation such that the mutant PDS is resistant to the herbicide R390244. The oligonucleotides may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalysed between the oligonucleotide and the target sequence. The oligonucleotide is introduced into tomato Mill cv H722 via microprojectile bombardment essentially as described by Eck *et al.* (1995 Plant Cell Reports

14, 299-304) and as outlined above for the other crops subjected to this transformation procedure.

Regenerable cotyledon explant material (as described by Fillati *et al.* (1997 Bio/technology 5 726-730) suspensions are bombarded with SEQ ID No. C oligonucleotide-coated particles by a helium-driven biolistics PDS 1000 system (BioRad) with a 300 mm Hg vacuum. The levels between the rupture disk and the macrocarrier and the macro-carrier and sample are varied for maximal transformation efficiency. Rupture disks of between 1000 and 2000 psi are used. The oligonucleotide may be introduced into the explant by multiple firings into the same tissue as necessary to optimise transformation efficiencies. The regenerable cotyledons are bombarded at the same stage as when *Agrobacterium* is used in the method of Beaudoin and Rothstein (1997 Plant Mol Biol 33 835 -846). Regeneration of tomato plants is as described by Beaudoin and Rothstein except that no selection agent is used. Primary putative transformants are grown in the greenhouse and cuttings are propagated in soil. These cuttings, once established, are sprayed with selecting concentrations of R390244 and allow transformed herbicide resistant plants to be identified. These transformed plants are grown to maturity and seeds resulting from self pollination are collected.

Mutation events in individuals is confirmed by amplifying the particular mutant gene sequence from herbicide resistant individuals spanning the region of mutation by PCR and sequencing individually isolated and cloned sequences.

Plants derived from material into which the oligonucleotides have been incorporated are resistant, more resistant or tolerant to the herbicide, when compared to plants derived from material not containing the said oligonucleotide.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:
(A) NAME: ZENECA LTD
(B) STREET: 15 Stanhope Gate
(C) CITY: LONDON
(E) COUNTRY: GB
10 (F) POSTAL CODE (ZIP): W1Y 6LN
(ii) TITLE OF INVENTION: IMPROVEMENTS IN OR RELATING TO ORGANIC COMPOUNDS
(iii) NUMBER OF SEQUENCES: 29
15 (iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
20 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1944 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
35 (iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Petunia hybrida
40 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 28..1578

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAATTCCTC AATCTTTACT TTCAAGA ATG GCA CAA ATT AAC AAC ATG GCT	51
Met Ala Gln Ile Asn Asn Met Ala	
1 5	
50 CAA GGG ATA CAA ACC CTT AAT CCC AAT TCC AAT TTC CAT AAA CCC CAA	99
Gln Gly Ile Gln Thr Leu Asn Pro Asn Ser Asn Phe His Lys Pro Gln	
10 15 20	
55 GTT CCT AAA TCT TCA AGT TTT CTT GTT TTT GGA TCT AAA AAA CTG AAA	147
Val Pro Lys Ser Ser Ser Phe Leu Val Phe Gly Ser Lys Lys Leu Lys	
25 30 35 40	
60 AAT TCA GCA AAT TCT ATG TTG GTT TTG AAA AAA GAT TCA ATT TTT ATG	195
Asn Ser Ala Asn Ser Met Leu Val Leu Lys Lys Asp Ser Ile Phe Met	
45 50 55	
65 CAA AAG TTT TGT TCC TTT AGG ATT TCA GCA TCA GTG GCT ACA GCA CAG	243
Gln Lys Phe Cys Ser Phe Arg Ile Ser Ala Ser Val Ala Thr Ala Gln	
60 65 70	

	AAG CCT TCT GAG ATA GTG TTG CAA CCC ATT AAA GAG ATT TCA GGC ACT	291
	Lys Pro Ser Glu Ile Val Leu Gln Pro Ile Lys Glu Ile Ser Gly Thr	
	75 80 85	
5	GTT AAA TTG CCT GGC TCT AAA TCA TTA TCT AAT AGA ATT CTC CTT CTT	339
	Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu	
	90 95 100	
10	GCT GCC TTA TCT GAA GGA ACA ACT GTG GTT GAC AAT TTA CTA AGT AGT	387
	Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Ser Ser	
	105 110 115 120	
15	GAT GAT ATT CAT TAC ATG CTT GGT GCC TTG AAA ACA CTT GGA CTG CAT	435
	Asp Asp Ile His Tyr Met Leu Gly Ala Leu Lys Thr Leu Gly Leu His	
	125 130 135	
20	GTA GAA GAA GAT AGT GCA AAC CAA CGA GCT GTT GTT GAA GGT TGT GGT	483
	Val Glu Glu Asp Ser Ala Asn Gln Arg Ala Val Val Glu Gly Cys Gly	
	140 145 150	
25	GGG CTT TTC CCT GTT GGT AAA GAG TCC AAG GAA GAA ATT CAA CTG TTC	531
	Gly Leu Phe Pro Val Gly Lys Glu Ser Lys Glu Glu Ile Gln Leu Phe	
	155 160 165	
30	CTT GGA AAT GCA GGA ACA GCA ATG CGG CCA CTA ACA GCA GCA GTT ACT	579
	Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr	
	170 175 180	
35	GTA GCT GGT GGA AAT TCA AGG TAT GTA CTT GAT GGA GTT CCT CGA ATG	627
	Val Ala Gly Gly Asn Ser Arg Tyr Val Leu Asp Gly Val Pro Arg Met	
	185 190 195 200	
40	AGA GAG AGA CCA ATT AGT GAT TTG GTT GAT GGT CTT AAA CAG CTT GGT	675
	Arg Glu Arg Pro Ile Ser Asp Leu Val Asp Gly Leu Lys Gln Leu Gly	
	205 210 215	
45	GCA GAG GTT GAT TGT TTC CTT GGT ACG AAA TGT CCT CCT GTT CGA ATT	723
	Ala Glu Val Asp Cys Phe Leu Gly Thr Lys Cys Pro Pro Val Arg Ile	
	220 225 230	
50	GTC AGC AAG GGA GGT CTT CCT GGA GGG AAG GTC AAG CTC TCT GGA TCC	771
	Val Ser Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser	
	235 240 245	
55	ATT AGC AGC CAA TAC TTG ACT GCT CTG CTT ATG GCT GCT CCA CTG GCT	819
	Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ala Ala Pro Leu Ala	
	250 255 260	
60	TTA GGA GAT GTG GAG ATT GAA ATC ATT GAC AAA CTA ATT AGT GTA CCT	867
	Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Val Pro	
	265 270 275 280	
65	TAT GTC GAG ATG ACA TTG AAG TTG ATG GAG CGA TTT GGT ATT TCT GTG	915
	Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Ile Ser Val	
	285 290 295	
70	GAG CAC AGT AGT AGC TGG GAC AGG TTC TTT GTC CGA GGA GGT CAG AAA	963
	Glu His Ser Ser Ser Trp Asp Arg Phe Phe Val Arg Gly Gly Gln Lys	
	300 305 310	
75	TAC AAG TCT CCT GGA AAA GCT TTT GTC GAA GGT GAT GCT TCA AGT GCT	1011
	Tyr Lys Ser Pro Gly Lys Ala Phe Val Glu Gly Asp Ala Ser Ser Ala	
	315 320 325	

	AGC TAC TTC TTG GCT GGT GCA GCA GTC ACA GGT GGA ACT ATC ACT GTT	1059
	Ser Tyr Phe Leu Ala Gly Ala Ala Val Thr Gly Gly Thr Ile Thr Val	
	330 335 340	
5	GAA GGT TGT GGG ACA AAC AGT TTA CAG GGG GAT GTC AAA TTT GCT GAG	1107
	Glu Gly Cys Gly Thr Asn Ser Leu Gln Gly Asp Val Lys Phe Ala Glu	
	345 350 355 360	
10	GTA CTT GAA AAA ATG GGA GCT GAA GTT ACG TGG ACA GAG AAC AGT GTC	1155
	Val Leu Glu Lys Met Gly Ala Glu Val Thr Trp Thr Glu Asn Ser Val	
	365 370 375	
15	ACA GTC AAA GGA CCT CCA AGG AGT TCT TCT GGG AGG AAG CAT TTG CGT	1203
	Thr Val Lys Gly Pro Pro Arg Ser Ser Ser Gly Arg Lys His Leu Arg	
	380 385 390	
20	GCC ATT GAT GTG AAC ATG AAT AAA ATG CCT GAT GTT GCC ATG ACA CTT	1251
	Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu	
	395 400 405	
25	GCT GTT GTT GCA CTT TAT GCT GAT GGT CCC ACA GCT ATA AGA GAT GTT	1299
	Ala Val Val Ala Leu Tyr Ala Asp Gly Pro Thr Ala Ile Arg Asp Val	
	410 415 420	
30	GCT AGC TGG AGA GTC AAG GAA ACT GAG CGC ATG ATC GCC ATA TGC ACA	1347
	Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr	
	425 430 435 440	
35	GAA CTT AGG AAG TTA GGA GCA ACC GTT GAA GAA GGA CCA GAC TAC TGC	1395
	Glu Leu Arg Lys Leu Gly Ala Thr Val Glu Glu Gly Pro Asp Tyr Cys	
	445 450 455	
40	ATA ATC ACC CCA CCG GAG AAA CTA AAT GTG ACC GAT ATT GAT ACA TAC	1443
	Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Asp Ile Asp Thr Tyr	
	460 465 470	
45	GAT GAT CAC AGG ATG GCC ATG GCT TTT TCT CTT GCT GCT TGT GCA GAT	1491
	Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp	
	475 480 485	
50	GTT CCC GTC ACC ATC AAT GAC CCT GGC TGC ACG CGG AAA ACC TTC CCT	1539
	Val Pro Val Thr Ile Asn Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro	
	490 495 500	
55	AAC TAC TTT GAT GTA CTT CAG CAG TAC TCC AAG CAT TGA ACCGCTTCCC	1588
	Asn Tyr Phe Asp Val Leu Gln Gln Tyr Ser Lys His	
	505 510 515	
60	TATATTGCAG AATGTAAGTA AGAATATGTG AAGAGTTTAG TTCTTGTACA AGACAGGCTA	1648
	CGACTGCCTG GTATCAGAAC CACAATGGGT TCCATTTTCAG TTCAGAAGGG CATTCCAAGG	1708
	CTTCGAACCTC TTTACTTATT TGCGAGTGAT GAAATGTATT TGTTAGAGTT GAGCTTCTTT	1768
	TTGTCTTTTAA GGAATGTACA CTAATAGAGT TAAGAATTAC TAGTATGGGC CAGTGTAAGG	1828
	AGTACTATTA CTCTTTGCTT ATTTTATTGA TTGAGTTTTC TCAAGGATCT GGCTTTGTCA	1888
	AGAATTACTG GTTAATTTTA TTGACAATCT CATGTGTCTA AATGAAATTG TTTGAT	1944

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

	Met	Ala	Gln	Ile	Asn	Asn	Met	Ala	Gln	Gly	Ile	Gln	Thr	Leu	Asn	Pro	
	1				5					10					15		
10	Asn	Ser	Asn	Phe	His	Lys	Pro	Gln	Val	Pro	Lys	Ser	Ser	Ser	Phe	Leu	
			20						25					30			
	Val	Phe	Gly	Ser	Lys	Lys	Leu	Lys	Asn	Ser	Ala	Asn	Ser	Met	Leu	Val	
			35					40					45				
15	Leu	Lys	Lys	Asp	Ser	Ile	Phe	Met	Gln	Lys	Phe	Cys	Ser	Phe	Arg	Ile	
		50					55					60					
	Ser	Ala	Ser	Val	Ala	Thr	Ala	Gln	Lys	Pro	Ser	Glu	Ile	Val	Leu	Gln	
20		65				70					75					80	
	Pro	Ile	Lys	Glu	Ile	Ser	Gly	Thr	Val	Lys	Leu	Pro	Gly	Ser	Lys	Ser	
					85					90					95		
25	Leu	Ser	Asn	Arg	Ile	Leu	Leu	Leu	Ala	Ala	Leu	Ser	Glu	Gly	Thr	Thr	
			100						105					110			
	Val	Val	Asp	Asn	Leu	Leu	Ser	Ser	Asp	Asp	Ile	His	Tyr	Met	Leu	Gly	
			115				120					125					
30	Ala	Leu	Lys	Thr	Leu	Gly	Leu	His	Val	Glu	Glu	Asp	Ser	Ala	Asn	Gln	
		130				135						140					
	Arg	Ala	Val	Val	Glu	Gly	Cys	Gly	Gly	Leu	Phe	Pro	Val	Gly	Lys	Glu	
35		145				150					155					160	
	Ser	Lys	Glu	Glu	Ile	Gln	Leu	Phe	Leu	Gly	Asn	Ala	Gly	Thr	Ala	Met	
					165					170					175		
40	Arg	Pro	Leu	Thr	Ala	Ala	Val	Thr	Val	Ala	Gly	Gly	Asn	Ser	Arg	Tyr	
			180						185					190			
	Val	Leu	Asp	Gly	Val	Pro	Arg	Met	Arg	Glu	Arg	Pro	Ile	Ser	Asp	Leu	
			195				200						205				
45	Val	Asp	Gly	Leu	Lys	Gln	Leu	Gly	Ala	Glu	Val	Asp	Cys	Phe	Leu	Gly	
		210				215						220					
	Thr	Lys	Cys	Pro	Pro	Val	Arg	Ile	Val	Ser	Lys	Gly	Gly	Leu	Pro	Gly	
50		225				230					235				240		
	Gly	Lys	Val	Lys	Leu	Ser	Gly	Ser	Ile	Ser	Ser	Gln	Tyr	Leu	Thr	Ala	
					245					250					255		
55	Leu	Leu	Met	Ala	Ala	Pro	Leu	Ala	Leu	Gly	Asp	Val	Glu	Ile	Glu	Ile	
			260						265					270			
	Ile	Asp	Lys	Leu	Ile	Ser	Val	Pro	Tyr	Val	Glu	Met	Thr	Leu	Lys	Leu	
			275				280						285				
60	Met	Glu	Arg	Phe	Gly	Ile	Ser	Val	Glu	His	Ser	Ser	Ser	Trp	Asp	Arg	
		290					295					300					
	Phe	Phe	Val	Arg	Gly	Gly	Gln	Lys	Tyr	Lys	Ser	Pro	Gly	Lys	Ala	Phe	
65		305				310					315					320	

Val Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala
 325 330 335

5 Val Thr Gly Gly Thr Ile Thr Val Glu Gly Cys Gly Thr Asn Ser Leu
 340 345 350

Gln Gly Asp Val Lys Phe Ala Glu Val Leu Glu Lys Met Gly Ala Glu
 355 360 365

10 Val Thr Trp Thr Glu Asn Ser Val Thr Val Lys Gly Pro Pro Arg Ser
 370 375 380

Ser Ser Gly Arg Lys His Leu Arg Ala Ile Asp Val Asn Met Asn Lys
 385 390 395 400

Met Pro Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Tyr Ala Asp
 405 410 415

20 Gly Pro Thr Ala Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr
 420 425 430

Glu Arg Met Ile Ala Ile Cys Thr Glu Leu Arg Lys Leu Gly Ala Thr
 435 440 445

25 Val Glu Glu Gly Pro Asp Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu
 450 455 460

Asn Val Thr Asp Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala
 465 470 475 480

Phe Ser Leu Ala Ala Cys Ala Asp Val Pro Val Thr Ile Asn Asp Pro
 485 490 495

35 Gly Cys Thr Arg Lys Thr Phe Pro Asn Tyr Phe Asp Val Leu Gln Gln
 500 505 510

Tyr Ser Lys His
 515

40 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Brassica napus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr
 1 5 10

65 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
5 (D) TOPOLOGY: unknown

 (ii) MOLECULE TYPE: peptide

 (iii) HYPOTHETICAL: NO
10
 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Brassica napus
15

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
20 Met Ala Ala Pro Leu Ala Leu Gly Asp Val Glu Ile
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:
25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 54 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
30 (D) TOPOLOGY: unknown

 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: synthetic
40

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
45 ATTGAGTTGT ACCTTGGGAA TGCAGGAACA GCCATGCGTC CACTCACC GC TGCA 54

(2) INFORMATION FOR SEQ ID NO: 6:
50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 39 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

55 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO
60
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: synthetic
65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAGUGGACGC AUGGCUGTTG CTGCAUCCCC AAGGUACAA

39

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ACTGCCCTCC TCATGGCAGC TCCTTTAGCT CTTGGAGACG TGGAGATTGA GATCATT

57

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAUCCACG UCUGCAAGAG TTAAAGGAGC UGCAUGAGG A

41

(2) INFORMATION FOR SEQ ID NO: 9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3831 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Brassica napus*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

5 AGATCTTAAA GGCTCTTTTC CAGTCTCACC TACCAAAACT ATAAGAAAAT CCACTTGCTG 60
TCTGAAATAG CCGACGTGGA TAAAGTACTT AAGACGTGGC ACATTATTAT TGGCTACTAG 120
10 AAAAAAAACT CATACACCAT CGTAGGAGTT GGGGTGGTG AAGAATTGA TGGGTGCCTC 180
TCCCCCCCCC ACTCACCAA CTCATGTTCT TTGTAAAGCC GTCACTACAA CAACAAAGGA 240
GACGACAGTT CTATAGAAAA GCTTTCAAAT TCAATCAATG GCGCAATCTA GCAGAATCTG 300
15 CCATGGCGTG CAGAACCCAT GTGTTATCAT CTCCAATCTC TCCAAATCCA ACCAAAACAA 360
ATCACCTTTC TCCGTCTCCT TGAAGACGCA TCAGCCTCGA GCTTCTTCGT GGGGATTGAA 420
20 GAAGAGTGGA ACGATGCTAA ACGGTTCTGT AATTCGCCCG GTTAAGGTAA CAGCTTCTGT 480
TTCCACGTCC GAGAAAGCTT CAGAGATTGT GCTTCAACCA ATCAGAGAAA TCTCGGGTCT 540
CATTAGCTA CCCGGATCCA AATCTCTCTC CAATCGGATC CTCCTTCTTG CCGCTCTATC 600
25 TGAGGTACAT ATACTTGCTT AGTGTTAGGC CTTTGCTGTG AGATTTTGGG AACTATAGAC 660
AATTTAGTAA GAATTTATAT ATAATTTTTT TAAAAAAAT CAGAAGCCTA TATATATTTA 720
30 AATTTTCCA AAATTTTGG AGGTTATAGG CTTATGTTAC ACCATTCTAG TCTGCATCTT 780
TCGGTTTGAG ACTGAAGAAT TTTATTTTTT AAAAAATTAT TATAGGGAAC TACTGTAGTG 840
GACAACTTGT TGAACAGTGA TGACATCAAC TACATGCTTG ATGCGTTGAA GAAGCTGGGG 900
35 CTTAACGTGG AACGTGACAG TGTAACAAC CGTGCGGTTG TTGAAGGATG CGGTGGAATA 960
TTCCAGCTT CCTTAGATTC CAAGAGTGAT ATTGAGTTGT ACCTTGGGAA TGCAGGAACA 1020
40 GCCATGCGTC CACTCACCGC TGCAGTTACA GCTGCAGGTG GCAACGCGAG GTAAGGTTAA 1080
CGAGTTTTTT GTTATTGTCA AGAAATTGAT CTTGTGTTTG ATGCTTTTAG TTGGTTTGT 1140
TTTCTAGTTA TGACTTGAT GGGGTGCCTA GAATGAGGGA AAGACCTATA GGAGATTTGG 1200
45 TTGTTGGTCT TAAGCAGCTT GGTGCTGATG TTGAGTGTAC TCTTGGCACT AACTGTCCTC 1260
CTGTTGCTGT CAATGCTAAT GGTGGCCTTC CCGGTGGAAG GGTGATCTTC ACATTTACTC 1320
50 TATGAATTGT TTGCAGCAGT CTTGTTCAT CACAGCCTTT GCTTCACATT ATTTTCATCTT 1380
TTAGTTTGTT GTTATATTAC TTGATGGATC TTTAAAAGG AATTGGGTCT GGTGTGAAAG 1440
TGATTAGCAA TCTTTCTCGA TTCCTGCGAG GGCCGTGGGC ATTACTAAGT GAAACATTAG 1500
55 CCTATTAACC CCCAAAATTT TTGAAAAAAA TTAGTATAT GGCCCCAAA TAGTTTTTTA 1560
AAAAATTAGA AAAACTTTTA ATAAATCGTC TACAGTCCCN NAAATCTTAG AGCCGGCCCT 1620
60 GCTGTATGG TTTCTGATT GATATATTAG ACTATGTTTT GAATTTTCAG GTGAAGCTTT 1680
CTGGATCGAT CAGTAGTCAG TACTTGACTG CCTCCTCAT GGCAGCTCCT TTAGCTCTTG 1740
GAGACGTGGA GATTGAGATC ATTGATAAAC TGATATCTGT TCCATATGTT GAAATGACAT 1800
65

	TGAAGTTGAT GGAGCGTTTT GGTGTTAGTG CCGAGCATAG TGATAGCTGG GATCGTTTCT	1860
	TTGTCAAGGG CGGTCAGAAA TACAAGTAAT GAGTTCTTTT AAGTTGAGAG TTAGATTGAA	1920
5	GAATGAATGA CTGATTAACC AAATGGCAAA ACTGATTGAG GTCGCCTGGT AATGCTTATG	1980
	TAGAAGGTGA TGCTTCTAGT GCTAGCTATT TCTTGGCTGG TGCTGCCATT ACTGGTGAAA	2040
10	CTGTTACTGT CGAAGGTTGT GGAACAACCTA GCCTCCAGGT AGTTTATCCA CTCTGAATCA	2100
	TCAAATATTA TTCTCCCTCC GTTTTATGTT AAGTGTCATT AGCTTTTAAA TTTTGTTC	2160
	TTAAAAGTGT CATTTTACAT TTTCAATGCA TATATTAAAT AAATTTTCCA GTTTTTACTA	2220
15	ATTCATTAAT TAGCAAAATC AAACAAAAAT TATATTAAAT AATGTAAAAT TCGTAATTTG	2280
	TGTGCAAATA CCTTAAACCT TATGAAACGG AAACCTTATG AAACAGAGGG AGTACTAATT	2340
20	TTATAATAAA ATTTGATTAG TTCAAAGTTG TGTATAACAT GTTTTGTAAG AATCTAAGCT	2400
	CATTCTCTTT TTATTTTTTG TGATGAATCC AAAGGGAGAT GTGAAATTCG CAGAGGTTCT	2460
	TGAGAAAATG GGATGTAAAG TGTCATGGAC AGAGAACAGT GTGACTGTGA CTGGACCATC	2520
25	AAGAGATGCT TTTGGAATGA GGCACCTGCG TGCTGTTGAT GTCAACATGA ACAAATGCC	2580
	TGATGTAGCC ATGACTCTAG CCGTTGTTGC TCTCTTGCC GATGGTCCAA CCACCATCAG	2640
30	AGATGGTAAA GCAAACCTT CTCTTTGAAT CAGCGTGTTT TAAAGATTTC ATGGTTGCTT	2700
	AAACTCTATT TGGTCAATGT AGTGGCTAGC TGGAGAGTTA AGGAGACAGA GAGGATGATT	2760
	GCCATTTGCA CAGAGCTTAG AAAGGTAAGT TTCCTTTTCT CTCATGCTCT CTCATTGAA	2820
35	GTTAATCGTT GCATAACTTT TTGCGGTTTT TTTTTTGCG TTCAGCTTG AGCTACAGTG	2880
	GAAGAAGGTT CAGATTATTG TGTGATAACT CCACCAGCAA AGGTGAAACC GCGGAGATT	2940
40	GATACGTATG ATGATCATAG AATGGCGATG GCGTTCTCGC TTGCAGCTTG TGCTGATGTT	3000
	CCAGTCACCA TCAAGGATCC TGGCTGCACC AGGAAGACTT TCCCTGACTA CTTCCAAGTC	3060
	CTTGAAAGTA TCACAAAGCA TTAAGGACC CTTTCCTCTG ATCCAAATGT GAGAATCTGT	3120
45	TGCTTTCTCT TTGTTGCCAC TGTAACATTT ATTAGAAGAA CAAAGTGTGT GTGTAAAGAG	3180
	TGTGTTTGCT TGTAATGAAC TGAGTGAGAT GCAATCGTTG AATCAGTTTT GGGCCTTAAT	3240
50	AAAGGGTTTA GGAAGCTGCA GCGAGATGAT TGTTTTTGAT CGATCATCTT TGAAAATGTG	3300
	TTTGTTTGAG TAATTTTTCT AGGGTTGAGT TGATTACACT AAGAAACACT TTTTGATTTT	3360
	CTATTACACC TATAGACACT TCTTACATGT GACACACTTT GTTGTTGGCA AGCAACAGAT	3420
55	TGTGGACAAT TTGCTTTTA ATGGAAGAA CACAGTTGTG GATGGGTGAT TTGTGGACGA	3480
	TTCCATGTGT GGTTAGGGTG ATTTGTGGAC GGATGATGTG TAGATGAGTG ATGAGTAATG	3540
60	TGTGAATATG TGATGTAAAT GTGTTTATAG TAGATAAGTG GACAACTCT CTGTTTTGAT	3600
	TCCATAAAAC TATACAACAA TACGTGGACA TGGACTCATG TTAATAAAAT TATACCGTAA	3660
	AACGTGGACA CGGACTCTGT ATCTCCAATA CAAACACTTG GCTTCTTCAG CTCAATTGAT	3720
65	AAATTATCTG CAGTTAAACT TCAATCAAGA TGAGAAAGAG ATGATATTGT GAATATGAGC	3780

GGAGAGAGAA ATCGAAGAAG CGTTTACCTT TTGTCGGAGA GTAATAGATC T

3831

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brassica napus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```

Met Ala Gln Ser Ser Arg Ile Cys His Gly Val Gln Asn Pro Cys Val
1      5      10      15
Ile Ile Ser Asn Leu Ser Lys Ser Asn Gln Asn Lys Ser Pro Phe Ser
20     25     30
Val Ser Leu Lys Thr His Gln Pro Arg Ala Ser Ser Trp Gly Leu Lys
35     40     45
Lys Ser Gly Thr Met Leu Asn Gly Ser Val Ile Arg Pro Val Lys Val
50     55     60
Thr Ala Ser Val Ser Thr Ser Glu Lys Ala Ser Glu Ile Val Leu Gln
65     70     75     80
Pro Ile Arg Glu Ile Ser Gly Leu Ile Lys Leu Pro Gly Ser Lys Ser
85     90     95
Leu Ser Asn Arg Ile Leu Leu Leu Ala Ala Leu Ser Glu Gly Thr Thr
100    105    110
Val Val Asp Asn Leu Leu Asn Ser Asp Asp Ile Asn Tyr Met Leu Asp
115    120    125
Ala Leu Lys Lys Leu Gly Leu Asn Val Glu Arg Asp Ser Val Asn Asn
130    135    140
Arg Ala Val Val Glu Gly Cys Gly Gly Ile Phe Pro Ala Ser Leu Asp
145    150    155    160
Ser Lys Ser Asp Ile Glu Leu Tyr Leu Gly Asn Ala Gly Thr Ala Met
165    170    175
Arg Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly Asn Ala Ser Tyr
180    185    190
Val Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Gly Asp Leu
195    200    205
Val Val Gly Leu Lys Gln Leu Gly Ala Asp Val Glu Cys Thr Leu Gly
210    215    220

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	Thr	Asn	Cys	Pro	Pro	Val	Arg	Val	Asn	Ala	Asn	Gly	Gly	Leu	Pro	Gly	
	225					230					235					240	
5	Gly	Lys	Val	Lys	Leu	Ser	Gly	Ser	Ile	Ser	Ser	Gln	Tyr	Leu	Thr	Ala	
					245					250						255	
	Leu	Leu	Met	Ala	Ala	Pro	Leu	Ala	Leu	Gly	Asp	Val	Glu	Ile	Glu	Ile	
				260					265					270			
10	Ile	Asp	Lys	Leu	Ile	Ser	Val	Pro	Tyr	Val	Glu	Met	Thr	Leu	Lys	Leu	
			275					280					285				
	Met	Glu	Arg	Phe	Gly	Val	Ser	Ala	Glu	His	Ser	Asp	Ser	Trp	Asp	Arg	
		290					295					300					
15	Phe	Phe	Val	Lys	Gly	Gly	Gln	Lys	Tyr	Lys	Ser	Pro	Gly	Asn	Ala	Tyr	
	305					310					315					320	
	Val	Glu	Gly	Asp	Ala	Ser	Ser	Ala	Ser	Tyr	Phe	Leu	Ala	Gly	Ala	Ala	
20					325					330					335		
	Ile	Thr	Gly	Glu	Thr	Val	Thr	Val	Glu	Gly	Cys	Gly	Thr	Thr	Ser	Leu	
				340					345					350			
25	Gln	Gly	Asp	Val	Lys	Phe	Ala	Glu	Val	Leu	Glu	Lys	Met	Gly	Cys	Lys	
			355					360					365				
	Val	Ser	Trp	Thr	Glu	Asn	Ser	Val	Thr	Val	Thr	Gly	Pro	Ser	Arg	Asp	
		370					375					380					
30	Ala	Phe	Gly	Met	Arg	His	Leu	Arg	Ala	Val	Asp	Val	Asn	Met	Asn	Lys	
	385					390					395					400	
	Met	Pro	Asp	Val	Ala	Met	Thr	Leu	Ala	Val	Val	Ala	Leu	Phe	Ala	Asp	
35					405					410					415		
	Gly	Pro	Thr	Thr	Ile	Arg	Asp	Val	Ala	Ser	Trp	Arg	Val	Lys	Glu	Thr	
				420					425					430			
40	Glu	Arg	Met	Ile	Ala	Ile	Cys	Thr	Glu	Leu	Arg	Lys	Leu	Gly	Ala	Thr	
			435					440					445				
	Val	Glu	Glu	Gly	Ser	Asp	Tyr	Cys	Val	Ile	Thr	Pro	Pro	Ala	Lys	Val	
		450					455					460					
45	Lys	Pro	Ala	Glu	Ile	Asp	Thr	Tyr	Asp	Asp	His	Arg	Met	Ala	Met	Ala	
	465					470					475					480	
	Phe	Ser	Leu	Ala	Ala	Cys	Ala	Asp	Val	Pro	Val	Thr	Ile	Lys	Asp	Pro	
50					485					490					495		
	Gly	Cys	Thr	Arg	Lys	Thr	Phe	Pro	Asp	Tyr	Phe	Gln	Val	Leu	Glu	Ser	
				500					505					510			
55	Ile	Thr	Lys	His													
				515													

(2) INFORMATION FOR SEQ ID NO: 11:

60

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 65 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
10 (A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

15 CTATGATCCC TAATGGTGGG GCTTTTTTAA GCCCACCATT AGGGAUCAUA GGCGCGTTTT 60
CGCGC 65

(2) INFORMATION FOR SEQ ID NO: 12:

20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
25 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
35 (A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

40 GTAATGCAGG AATAGCAATG CGTCCTTTTG GACGCAUUGC TATTCUGCA UUACGCGCGT 60
TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 13:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
50 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

55 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

5 GTAATGCAGG AATAGCAATG CGTTCCTTTTG AACGCAUUGC TATTCCTGCA UUACGCGCGT 60
 TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 14:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: circular

15 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: oligonucleotide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

 ACAGCAATGC GTTCACTTAC CGCTGTTTTTC AGCGGUAAGT GAACGCAUUG CUGUGCGCGT 60
 TTCGCGC 67

30 (2) INFORMATION FOR SEQ ID NO: 15:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 base pairs
35 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: circular

40 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

45 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: oligonucleotide

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

50 ATAGCAATGC GTTCACTTAC CGCTGTTTTTC AGCGGUAAGT GAACGCAUUG CUAUGCGCGT 60
 TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 16:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 89 base pairs
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

5 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

15 GTAATGCAGG AATAGCAATG CGTTCACTCA CCGCTGTTTT CAGCGGUGAG TGAACGCAUU 60

GCTATTCCUG CAUUACGCGC GTTTCGCGC 89

20 (2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 67 base pairs

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: both

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

35 (A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGAATGCAGG AATAGCCATG CGTCCTTTTG GACGCAUCGC TATTCCUGCA UCCGCGCGT 60

40 TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH: 67 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: circular

50 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

55 (vi) ORIGINAL SOURCE:

(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

5 GGAATGCAGG AATAGCCATG CGTTCTTTTG AACGCAUCGC TATTCCTGCA UUCCGCGCGT 60
TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 19:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

15 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: oligonucleotide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ACAGCCATGC GTTCACTCAC CGCTGTTTTTC AGCGGUGAGT GAACGCAUGG CUGUGCGCGT 60
30 TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 20:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

40 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (vi) ORIGINAL SOURCE:
(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

50 ATAGCCATGC GTTCACTCAC CGCTGTTTTTC AGCGGUGAGT GAACGCAUGG CUAUGCGCGT 60
TTCGCGC 67

55 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

5 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

10 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: oligonucleotide

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

15 GGAATGCAGG AATAGCCATG CGTTCACTCA CCGCTGTTTT CAGCGGUGAG TGAACGCAUC 60

 GCTATTCCUG CAUUCGCGC GTTTCGCGC 89

20 (2) INFORMATION FOR SEQ ID NO: 22:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71 base pairs
 (B) TYPE: nucleic acid
25 (C) STRANDEDNESS: both
 (D) TOPOLOGY: circular

 (ii) MOLECULE TYPE: other nucleic acid

30 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
35 (A) ORGANISM: oligonucleotide

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

 GGAATGCTGG AATCGCAATG CGGCCATTTT TAUGGCCGCA UUGCGATTCC AGCAUCCGC 60

40 GCGTTTCGCG C 71

 (2) INFORMATION FOR SEQ ID NO: 23:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
50 (D) TOPOLOGY: circular

 (ii) MOLECULE TYPE: other nucleic acid

 (iii) HYPOTHETICAL: NO

55 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

5 GGAATGCTGG AATCGCAATG CGGTCATTTT TAUGACCGCA UUGCGATTCC AGCAUCCGC 60
GCGTTTCGCG C 71

(2) INFORMATION FOR SEQ ID NO: 24:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
15 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: oligonucleotide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTGCAATGCG GTCATTGACA GCAGCTTTTG CUGCUGUCAA TGACCGCAUU GGCAGGCGCG 68
TTTCGCGC

30

(2) INFORMATION FOR SEQ ID NO: 25:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

40 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45

(vi) ORIGINAL SOURCE:
(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

50

TCGCAATGCG GTCATTGACA GCAGCTTTTG CUGCTGUCAA TGACCGCAUU GCGAGCGCGT 60
TTTCGCGC 67

55 (2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: circular

5 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

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GGAAATGCTGG AATCGCAATG CGGTCATTGA CAGCAGCTTT TGCUGCUGUC AATGACCGCA 60

UUGCGATTCC AGCAUUCGCG GCGTTTCGCG C 91

20 (2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 67 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: both

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

40 TCGCATTGAA CAGCTTTCTT CAGGTTTTTA CCUGAAGAAA GCTGUUCAAU GCGAGCGCGT 60

TTCGCGC 67

(2) INFORMATION FOR SEQ ID NO: 28:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

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(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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TTGTACCTTG GGAATGCAGG AACAGCCATG CGTCCACTC

39

(2) INFORMATION FOR SEQ ID NO: 29:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

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(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: oligonucleotide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

TCCTCATGGC AGCTCCTTTA GCTCTTGGAG ACGTGGAGAT T

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CLAIMS

1. A method of producing plants which exhibit an agronomically desirable trait comprising mutating or otherwise modifying *in situ* in a plant cell at least one gene which when modified is responsible for providing the said trait and regenerating from a cell exhibiting the said trait fertile morphologically normal whole plants, characterised in that a polynucleotide is introduced into the plant cell, the said polynucleotide comprising at least one region which is substantially complementary to at least one region in the gene, which gene region when mutated or otherwise modified provides for the agronomically desirable trait, the region in the said polynucleotide containing at least one base mismatch in comparison with the like region in the said gene, so that the region in the said gene is altered by the DNA repair/replication system of the cell to include the said mismatch.
2. A method according to the preceding claim, wherein - prior to the *in situ* mutation or modification, the plant cell is transformed with a gene providing for an agronomically desirable trait, and/or the cell is treated with a chemical mutagen.
3. A method according to either of claims 1 or 2, wherein at least one of the following regions of the gene is mutated or otherwise modified: promoter, RNA encoding sequence or transcription terminator.
4. A method according to any preceding claim, wherein the transcription activating region of the gene is mutated or otherwise modified *in situ*.
5. A method according to any preceding claim, wherein the said trait is herbicide resistance.
6. A method according to the preceding claim, wherein the herbicide is selected from the group consisting of paraquat; glyphosate; glufosinate; photosystem II inhibiting herbicides; dinitroaniline or other tubulin binding herbicides; herbicides which inhibit imidazole glycerol phosphate dehydratase; herbicides which inhibit

acetolactate synthase; herbicides which inhibit acetyl CoA carboxylase; herbicides which inhibit protoporphyrinogen oxidase; herbicides which inhibit phytoene desaturase; herbicides which inhibit hydroxyphenylpyruvate dioxygenase and herbicides which inhibit the biosynthesis of cellulose.

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7. A method according to any one of claims 2 to 6, wherein the plant cell is prior transformed with a gene providing for resistance to insects, fungi, and/or herbicides.

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8. A method according to any preceding claim, wherein the protein encoding region of the gene encodes an enzyme selected from the group consisting of EPSPS, GOX, PAT, HPPD, ACC, ALS, BNX and protox.

9. A method according to the preceding claim, wherein the said at least one region of the polynucleotide consists of RNA.

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10. A method according to the preceding claim, wherein the polynucleotide other than that comprised by the said at least one region consists of DNA.

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11. A method according to any one of the preceding claims, wherein the polynucleotide consists of between about 30 and 250 nucleotides.

12. A method according to the preceding claim, wherein the polynucleotide consists of between 50 and 80 nucleotides.

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13. A method according to any preceding claim, wherein the polynucleotide comprises between about 60 and about 150 bases and has an overall 'dumbbell' like shaped secondary structure looped around upon itself at either end and with a central 'rod' region of paired complementary DNA and RNA sequences.

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14. A method according to any one of claims 8 to 13, in which the said gene encodes an EPSPS having at least the residues Thr, Pro, Gly and Ala at positions corresponding to 174, 178, 173 and 264 with respect to the EPSPS depicted in SEQ ID No. 2,

wherein the said mismatch results in at least one of the following modifications in the EPSPS enzyme in comparison with the native sequence:

- (i) Thr 174 - Ile
- (ii) Pro 178 - Ser
- (iii) Gly 173 - Ala
- (iv) Ala 264 - Thr

wherein (i) Thr 174 occurs within a sequence comprising contiguously Ala -Gly-Thr-Ala-Met; (ii) Pro 178 occurs within a sequence comprising contiguously Met-Arg-Pro-Leu-Thr; (iii) Gly 173 occurs within a sequence comprising contiguously Asn-Ala-Gly-Thr-Ala; and (iv) Ala 264 occurs within a sequence comprising contiguously Pro-Leu-Ala-Leu-Gly.

- 15. A method according to any one of claims 8 to 14, wherein the mismatch results in replacement of the terminal Gly residue within the sequence motif Glu-Arg-Pro-AA1-AA2-AA3-Leu-Val-AA4-AA5-Leu-AA6-AA7-AA8-Gly- in a region of the EPSPS enzyme corresponding to that spanning positions 202 to 216 in SEQ ID No. 2 by either an Asp or Asn residue.
- 16. A method according to any preceding claim, wherein the plant cell is a cell of a plant selected from the group consisting of canola, sunflower, tobacco, sugar beet, cotton, maize, wheat, barley, rice, sorghum, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, cabbage, onion, soya spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and forage grasses, flax and oilseed rape, and nut producing plants insofar as they are not already specifically mentioned.
- 17. A method according to any preceding claim, wherein the plant cell is converted into a protoplast prior to the *in situ* mutation or modification of the gene, or transcriptional activating regions thereof, which when modified provides for the agronomically desirable trait.
- 18. Plants which result from the method of any preceding claim, the progeny and seeds of such plants, and plant material derived from such plants, progeny and seeds.

19. A method of controlling weeds in a field, the field comprising weeds and plants according to claim 18, the method comprising application to the field of a herbicide to which the said plants have been rendered resistant.

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20. A method according to the preceding claim, further comprising the steps of applying to the field insecticidally effective amounts of insecticides and/or fungicidally effective amounts of fungicides after the field has been treated with the herbicide.



Application No: GB 9811138.8
Claims searched: 1-20

Examiner: Dr Jon Broughton
Date of search: 6 October 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): C3H (HB7T)

Int Cl (Ed.6): C12N 15/82

Other: ONLINE: DIALOG/BIOTECH, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	Mol. Gen. Genet. (1992) 231 186-193 (HALFTER U. <i>et al.</i>) See whole document, particularly figure 1.	1-3, 5-7 and 16-18.
X	Plant Cell (1990) 2 (5) 415-425 (LEE K. Y. <i>et al.</i>) See whole document, particularly plasmid pAGS 182BV (figure 1) and "Results" from page 416 to page 419.	1, 3, 5, 6, 8 and 16-18.

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.